

## AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior versions, and listings, of claims in the present application.

### LISTING OF THE CLAIMS:

1-30 (canceled)

31. (currently amended) A method of isolating at least one anti-ligand to at least one target ligand comprising the steps of:

- (i) providing a library of anti-ligands;
- (ii) providing a first population of ligands comprising a ligand fixed to or incorporated in a subtractor ligand construct wherein said subtractor ligand construct comprises a subtractor ligand associated with a separation means;
- (iii) providing a second population of ligands comprising the same ligand as step (ii), fixed to or incorporated in a target ligand construct wherein said target ligand construct comprises a target ligand associated with a separation means;
- (iv) determining amounts of subtractor ligand construct and target ligand construct using one or more equations derived from the universal law of mass action  $\frac{[C]^c[d]^d}{[A]^a[B]^b} = K_{eq}$ ,  
where:  
A, B, C & D = are the participants in the reaction (reactants and products)  
a, b, c, & d = the coefficients necessary for a balanced chemical equation to permit the determination of the amount of subtractor ligand as compared to target ligand required for isolation of at least one anti-ligand to at least one target ligand;
- (v) providing the amount of the subtractor ligand construct determined in step (iv);
- (vi) providing the amount of the target ligand construct determined in step (iv);
- (vii) providing separation means for isolating anti-ligand bound to the target ligand construct from anti-ligand bound to the subtractor ligand construct, wherein said separation means for the subtractor and target ligand constructs can be either the same or different;

(viii) exposing the library of (i) to the ligands of (v) and (vi) to permit binding of anti-ligands to ligands; and

(ix) isolating the anti-ligand bound to the ligand fixed to or incorporated in the target ligand construct ~~using~~ utilizing the separation means.

32-33 (canceled)

34. (previously presented) A method as claimed in claim 31 comprising a further step of releasing the anti-ligand from the ligand.

35. (previously presented) A method as claimed in claim 31 whereby steps (ii) to (ix) are repeated one or more times.

36. (previously presented) A method as claimed in claim 31 wherein the amount of one of the subtractor ligand construct or target ligand construct is provided in excess of the amount of the other of the subtractor ligand construct or the target ligand construct.

37. (previously presented) A method as claimed in claim 36 where the excess of ligand is of 10 to 100 fold.

38. (previously presented) A method as claimed in claim 31 wherein the equation of (iv) is

$$bA = \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT}$$

where

bA = Bound anti-ligand

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant ( $6.022 \times 10^{23}$  particles/mole)

V = Reaction volume (litres)

$K_d$  = Equilibrium dissociation constant.

39. (previously presented) A method as claimed in claims 31 wherein the equation of (iv) is:

$$bA = \left\{ \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT} \right\} x \left\{ \frac{(T_p x C_p)}{((T_p x C_p) + (T_s x C_s))} \right\}$$

where

$bA_p$  = Bound anti-ligand

$T_p$  = The number of ligands on  $C_p$

$T_s$  = The number of ligands on  $C_s$

$C_p$  = The number of target ligand constructs

$C_s$  = The number of subtractor ligand constructs

$A$  = Total number of anti-ligand

$T$  = Total number of ligands

$C$  = Avogadro's constant ( $6.022 \times 10^{23}$  particles/mole)

$V$  = Reaction volume (litres)

$K_d$  = Equilibrium dissociation constant.

40. (previously presented) A method as claimed in claim 31 wherein the separation means are selected from at least one of a solid support, cell membrane and/or portions thereof, synthetic membrane, beads, chemical tags and free ligand.

41. (previously presented) A method as claimed in claim 40 whereby the separation means are cell membranes and/or portions thereof.

42. (previously presented) A method as claimed in claim 41 whereby the subtractor and target ligands are fixed to and/or incorporated within separate cell membranes and/or portions thereof.

43. (previously presented) A method as claimed in claim 31 whereby the separation means of the subtractor and target ligand constructs have a different density.

44. (previously presented) A method as claimed in claim 43 wherein the separation means of the subtractor ligand construct is of a lower density than the separation means of the target ligand construct.

45. (previously presented) A method as claimed in claim 44 wherein the separation means of the subtractor ligand construct is a membrane vesicle.

46. (previously presented) A method as claimed in claim 44 wherein the separation means of the target ligand construct is a whole cell membrane.

47. (previously presented) A method as claimed in claim 31 whereby step (ix) is performed by at least one of density centrifugation, solid support sequestration, magnetic bead sequestration, chemical tag binding and aqueous phase partitioning.

48. (previously presented) A method as claimed in claim 47 whereby the isolation step is performed by density centrifugation.

49. (previously presented) A method as claimed in claim 48 wherein the density centrifugation is performed using a sucrose-polymer gradient.

50. (previously presented) A method as claimed in claim 31 wherein the library of step (i) is a display library comprising a plurality of library members which display anti-ligands.

51. (previously presented) A method as claimed in claim 50 wherein the library is a phage display library.

52. (previously presented) A method as claimed in claim 31 wherein the subtractor and target ligands are independently at least one from antigens; receptor ligands; and enzyme targets that comprise at least one selected from carbohydrate; protein; peptide; lipid; polynucleotide; inorganic molecules and conjugated molecules.

53. (previously presented) A method as claimed in claim 31 wherein the library of anti-ligands is composed of at least one selected from antibodies, and antigen binding variants, derivatives or fragments thereof; scaffold molecules with engineered variable surfaces; receptors; and enzymes.

54. (previously presented) A method as claimed in claim 31 comprising a further step of exposing the ligand and its separation means to a stimulus which influences the expression of target ligands on said ligand constructs.

55-59 (canceled)